1. INTRODUCTION

In this paper a new approach is made to the problem of the biological action of ions, particularly to the large class of antagonistic or balanced actions such as the antagonistic action of sodium and calcium which is important in determining cell permeability, in the behaviour of the heart, in the survival of marine organisms in artificial media, for the optimum development of plants, etc. In these balanced actions it is common for the action of one calcium ion to be balanced by the action of 50-100 sodium ions.† It is very difficult to see what kind of physico-chemical mechanism can enable one calcium ion to offset the action of 25 or more sodium ions. The modern theory of strong electrolytes in homogeneous aqueous solution has completely failed to provide any clue for the solution of this problem. However, the situation would be much simplified if it could be shown that at the actual site of action of the ions the ionic ratio is not of the order of 50:1, as in the environmental fluid, but more nearly approaches 1:1.

There is much evidence to suggest that the actual sites upon which such ions as calcium exert their action are surfaces, particularly the cell surface and the surfaces of protein molecules. These surfaces are usually charged. It has long been implicit in the theory of colloid chemistry that the concentration of ions in the immediate vicinity of a charged surface is substantially different from the concentrations in the media bathing these surfaces, and in several papers (Danielli, 1937, 1941; Hartley & Roe, 1940) it has been clearly demonstrated that in particular the hydrogen-ion concentration at a surface may be up to 10 or even 100 times different from that in the surrounding media. In these papers methods have been developed for calculating the concentrations of ions in the interior of the electrical double layer of a surface. By applying these methods to biological systems we can therefore calculate the actual concentrations of ions at the surfaces upon which the ions act. Wilbrandt (1939) has previously shown that consideration of the Donnan equilibrium between the cell surface and the environmental fluid leads to significant relationships dealing with the relative action of sodium and calcium upon the permeability of the red cell to water and to potassium.‡

2. THE CALCULATION OF ION CONCENTRATIONS AT SURFACES

There are at present two methods of calculating the concentrations of ions at a surface. In the first place (Danielli, 1937, 1941) we may regard the surface as constituting a distinct phase and calculate the distribution of ions between the environmental phase and the surface phase from the Gibbs-Donnan equilibrium. If the various ionic species considered in any one phase all have the same activity coefficient, or if a given ion has the same activity coefficient in both phases, then we obtain

\[ (\text{pH})_s - \text{pH}_b = \log \frac{[\text{Na}]_s}{[\text{Na}]_b} = \log \frac{[\text{Cl}]_s}{[\text{Cl}]_b} = \text{etc.} \]

Here the suffixes \(s\) and \(b\) denote surface and bulk (environmental) phases respectively and the suffix \(D\) denotes a pH or concentration calculated in this way.

The second method of calculating the surface concentration of ions is that of Hartley & Roe (1940), who have shown that the concentrations may be calculated from the electrokinetic potential, \(\zeta\), using the equation

\[ (\text{pH})_s - \text{pH}_b = \zeta/60 = 0.325u = -\log \frac{[\text{Na}]}{[\text{Na}]_s^2} = \text{etc.} \]

† I have previously discussed with Dr Wilbrandt some of the points made in this paper concerning calcium, and greatly regret that the cutting of communications with Switzerland has prevented our pursuing this enquiry together.

‡ Beit Memorial Fellow and Fellow of St John's College, Cambridge.

* Beit Memorial Fellow and Fellow of St John's College, Cambridge.

† For the normal functioning of many tissues a sodium-potassium ratio of about 30:1 is required, but it seems probable that the action of potassium is mainly important in determining the resting potential and the internal potassium concentration of the cells, so that its mode of action is considerably different from that which will be proposed for such ions as calcium.
for small particles at 25°C. For large particles such as cells the corresponding expression is
\[(pH)_b - pH = \frac{\log c}{RT} E\]
The suffix \(E\) denotes a \(pH\) or concentration calculated from (2) or (3). \(u\) is the mobility in \(\mu\text{sec.}/\text{V.}/\text{cm}^2\).

It has been shown that the distribution of hydrogen ions between the surface of ovalbumin molecules and the bulk phase is the same whichever of these two methods is used (Danielli, 1941). The experimental data required for the first method are the ionic composition of the bulk phase, the net number of positively or negatively charged groups fixed on the surface of the protein molecule, and the total surface area of the protein molecule. The data required by the second method are the ionic composition of the bulk phase and the electrokinetic mobility or potential.

Let us now consider the concentrations of sodium and calcium ions at the surface of the protein molecule. Some of the necessary information is lacking for serum proteins, so we shall make this calculation for ovalbumin. The hydrogen-ion concentration at the surface of ovalbumin molecules has been given elsewhere (Danielli, 1941). Let \(C_1\) be the total concentration in electrical equivalents of sodium and calcium ions in the surface of an ovalbumin molecule. Then we have, if \([H^+]\) is small,
\[\frac{[Na^+]_b}{[Ca^{++}]_b} = \frac{2[Ca^{++}]}{[Na^+]_b} + [Na^+]_b = C_i\text{ equivalents.} \tag{4}\]

Also, from the Gibbs-Donnan relationship, we have
\[\frac{[Na^+]_b}{[Ca^{++}]_b} = \frac{[Na^+]_b}{[Ca^{++}]_b} \cdot \frac{1}{\frac{1}{2}[Ca^{++}]_b + [Na^+]_b} \tag{5}\]

Solving these two simultaneous equations we have
\[\frac{[Na^+]_b}{[Ca^{++}]_b} = \frac{2[Ca^{++}]}{[Na^+]_b} = \frac{1}{\frac{1}{2}[Ca^{++}]_b + [Na^+]_b} \tag{6}\]

All the quantities on the right-hand side of this last equation can be measured experimentally. The method of calculating \(C_i\) is given by Danielli (1941). Having obtained \([Na^+]_b\), from this equation we can most conveniently calculate \([Ca^{++}]_b\), from equation (5). Table 1 shows results calculated in this way for ovalbumin molecules in an environment 0.133 M NaCl and 0.004 M CaCl₂. This corresponds to the concentrations used by R. F. Loeb in a systematic study of the binding of calcium by egg albumin (1925). The last column of the table contains the ratio of sodium and calcium ions in the surface. Whereas in the environment this ratio is always 25:1, in the surface the ratio varies from 113:1, when the protein is positively charged at pH 2.8, to 17:1 when the protein is negatively charged at pH 7.5. Thus, on the alkaline side, there is a binding of calcium at the surface of the ovalbumin molecules due to the fixed negative charge of the molecules. From the results in Table 1 we can calculate the amount of calcium which is bound in this way in a protein solution containing a known percentage of protein. R. F. Loeb used a 6-2% solution of ovalbumin. This contains 6.2 x 10⁻⁴ M of ovalbumin per 100 c.c. of solution. The volume of the surface phase of one molecule of ovalbumin in 0.133 M NaCl is given by Danielli (1941) as 1.08 x 10⁻¹⁴ c.c. Consequently the total volume of surface phase per 100 c.c. of solution is
\[1.08 x 10^{-14} M \times 6.6 x 10^{-4} M \times 10^3 = 9.7 \text{ c.c.}\]

Hence, the protein solution may be regarded as made up of 97% of solution having the composition given in Table 1 for the surface phase and of (100 - 97)% of the environmental fluid. Since on the alkaline side of the isoelectric point there is an excess of calcium in the surface, the ratio, \([Ca^{++}]_b\) in ovalbumin solution : \([Ca^{++}]_b\) in environmental fluid, will be greater than unity, and on the acid side of the isoelectric point, the ratio will be correspondingly less than unity. The crosses on Fig. 1 show the calculated values of this ratio, using the information of Table 1. The open circles show the values of a similar ratio obtained by Loeb (1925) by experimental methods. It is evident that ovalbumin actually binds far more calcium than is indicated by the calculation just made, i.e. the amount of calcium which may be bound by the electrical field of the net electrical charge of the ovalbumin molecule is insufficient to account for the total binding of calcium found experimentally. In other words, part of the calcium bound by ovalbumin is behaving as though it formed an undissociated complex with some group in the ovalbumin surface.

It is not immediately clear with which groups of the protein molecule calcium is likely to form unionized compounds. But when the titration curve of ovalbumin (see Cannan, 1938) is compared with the results of Loeb, it is found that the amount of calcium binding increases rapidly as the COOH groups of the protein are converted into COO⁻. This suggests that calcium is entering into reaction such as
\[Ca^{++} + COO^- \rightleftharpoons Ca^{++} COO. \tag{7}\]
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Let us assume that this reaction is the one responsible for the binding of calcium over and above that bound by the electrostatic effect. Then we can write for this reaction

\[
\frac{[\text{Ca}^{++}]_a [\text{COO}^-]_a}{[\text{Ca}^{+}\text{COO}^-]_a} = k,
\]

where \( k \) is a constant. It should be noted that this constant is not the same as the somewhat similar dissociation constants for calcium proteinates obtained by McLean & Hastings (1935) and Greenberg & Power (1931), who were using bulk and not surface concentrations. If we now evaluate

\[
[\text{COO}^-]_a + [\text{Ca}^{+}\text{COO}^-]_a = \frac{1000}{1.08 \times 10^{-19}} = 0.76 M \text{ at pH 7.2.}
\]

Similarly from the analyses of Loeb we can calculate that the total calcium concentration at the surface of the ovalbumin molecule at pH 7.2 is 0.0312, and from calculations such as those of Table 1 we find that \([\text{Ca}^{++}]_a = 0.015 M\). The difference between these two figures, i.e. 0.0162, must be the concentration of \( \text{Ca}^{+}\text{COO}\). Hence

\[
k = \frac{0.015 \times 0.73}{0.0162} = 0.69.
\]

Having obtained the value of \( k \) we can now calculate the amount of calcium bound by this mechanism at other pH values. The results of such calculations are given in Table 2. For each pH value it was necessary first to obtain the value of \([\text{Ca}^{++}]_a\), as in Table 1, then the value of \([\text{COO}^-]_a\), from the number of ionized carboxyl groups per molecule at the given pH, and then \([\text{Ca}^{+}\text{COO}]_a\) can be calculated from equation (8). The final column of Table 2 shows the ratio of the total sodium concentration in the surface to the total calcium concentration in the surface. It will be seen that at pH 7.2 this ratio is reduced to 7.5, compared with a value of 33 in the environment. The closed circles of Fig. 1 show values calculated for the binding of calcium by a 6.2% ovalbumin solution at various pH, calculated from the data of Table 2 by the same method as used before, but in this case including both the calcium bound in the unionized form and the excess of calcium present as a result of the Donnan equilibrium between the surface and the environment. These points are in good agreement with the experimental values obtained by Loeb. We may therefore conclude that the calcium bound by ovalbumin is bound in two ways: partly as the result of the

<table>
<thead>
<tr>
<th>pH</th>
<th>([\text{Ca}^{++}]_a)</th>
<th>([\text{COO}^-]_a+[\text{Ca}^{+}\text{COO}]_a)</th>
<th>([\text{Ca}^{+}\text{COO}]_a)</th>
<th>([\text{Na}^+]_a)</th>
<th>([\text{Na}^+]_a+[\text{Ca}^{++}]_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>0.0009</td>
<td>0.38</td>
<td>0.005</td>
<td>0.064</td>
<td>46</td>
</tr>
<tr>
<td>5.7</td>
<td>0.0067</td>
<td>0.69</td>
<td>0.0067</td>
<td>0.171</td>
<td>13</td>
</tr>
<tr>
<td>6.5</td>
<td>0.011</td>
<td>0.72</td>
<td>0.011</td>
<td>0.22</td>
<td>10</td>
</tr>
<tr>
<td>7.2</td>
<td>0.015</td>
<td>0.76</td>
<td>0.0162</td>
<td>0.24</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Table 2. Calculation of the molar concentration of ionic and of undissociated calcium at the surface of ovalbumin molecules in equilibrium with an environment containing 0.133 M NaCl + 0.004 M CaCl₂.
Gibbs-Donnan equilibrium between the surface of the protein molecules and the environment, and partly as an apparently undisassociated complex between ionized carboxyl groups and calcium.

A somewhat similar finding was obtained by Webb & Danielli (1940) for the binding of calcium by palmitate monolayers. Monolayers of palmitic acid were spread on alkaline solutions containing various concentrations of sodium and calcium. These monolayers were scraped off the surface after they had come into equilibrium with the bulk phase, and their sodium and calcium content determined. It was found that, as the Donnan equilibrium between surface and bulk phase requires, the sodium: calcium ratio in the monolayer is much less than in

(a) Calculation from \( \xi \) potential. Calculations are given in Table 3 for various cells using equation (3). The experimental values of \( \xi \) are those given by Abramson (1934), Heilbrunn (1937) and Mudd, Mudd & Keltch (1929). The values of the concentration of ionic calcium given in the sixth column of Table 3 are very considerably higher than the ionic calcium concentrations in the environment given in column 3 of the same table. In addition it is necessary to take some account of the unionized calcium present at the surfaces of these cells. The

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
</table>
| Red cell (rat) | 0.145 | 0.0036 | 0.15 | 0.26 | 0.01 | 0.03 | 45 | 2 
| Red cell (horse) | 0.145 | 0.0043 | 0.35 | 0.25 | 0.0087 | 0.09 | 45 | 2.8 |
| Arteria sperm | 0.145 | 0.003 | 0.019 | 0.00015 | 0.08 | 0.09 | 45 | 3.8 |
| Arteria sperm | 0.145 | 0.003 | 0.019 | 0.00015 | 0.08 | 0.09 | 45 | 3.8 |
| Arteria sperm | 0.145 | 0.003 | 0.019 | 0.00015 | 0.08 | 0.09 | 45 | 3.8 |
| Cummingia egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Arbacia egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Arbacia egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Arbacia egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Arbacia egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Echinorachnius egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Echinorachnius egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Echinorachnius egg | 0.05 | 0.02 | 0.20 | 0.20 | 0.20 | 0.20 |
| Tubercle bacilli | 0.00023 | 0.00006 | 35 | 0.00088 | 0.000086 | 0.0009 | 50 | 1.0 |

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in column 7 of Table 3. Of course, this constitutes only a very rough estimate and is not intended to do more than indicate the order of magnitude of the unionized calcium concentration which studies of artificial systems lead us to expect. It is interesting to see that the sodium:calcium ratio for the surface of these cells given in column 9 of Table 3 differs by a comparatively small amount from unity. Thus, our provisional calculations of ion concentrations at the surfaces of cells which are bathed by a physiologically balanced saline suggest that, whereas the sodium:calcium ratio in the saline is of the order of 25:1 or more, the ratio at the surface of the cell, where the balanced action presumably takes place, is approximately unity. This very strongly suggests that the reason for the efficacy of a ratio of the order of 25:1 in physiological saline is that it is this ratio in the bulk phase which is required to give a physico-chemically balanced ratio of the order of 1:1 at the site of action of the ions, i.e., at the cell surface. Some of the results presented in Table 3 for Echinoderm sperm were obtained with the sperm in calcium-free media. The value of [Na⁺], obtained in this medium is and Elodea cells must be regarded with some suspicion, because these cells are surrounded by a rigid framework which probably also contributes to the binding of calcium. But the value for Arbacia eggs is probably substantially correct. It is therefore interesting to see that the figure of 1.64 M, which corresponds to one calcium atom per 230 Å³, is in reasonable agreement with the estimate of 2.0 M given in Table 3 for the total calcium concentration at the surface of Arbacia eggs.

Further evidence of the binding of ions at cell surfaces was obtained by Mazia & Mullins (1941), using radioactive copper. It was shown that the concentration of copper at the cell surface could rise by a factor of 10⁶ or more above the concentration in the environment. This radioactive copper was so firmly bound that it could hardly be removed by washing with saline, but on the other hand it would exchange almost instantaneously with solutions containing either non-radioactive copper, or divalent gold, thus showing that the radioactive copper had entered into a complex with the cell surface which was in dynamic equilibrium with other ions.

(c) Calculation from lipid analysis. Many workers

have shown that practically all of the lipid present in red blood cells must be present in the cell membrane and that even so this membrane can only be about 2 lipid molecules thick. These lipid molecules must be oriented with their polar groups towards the lipid-water interfaces of the membrane (see Danielli, 1942). Consequently, from simple quantitative analysis of red cell lipid it is possible to obtain a measure of the number of potentially available ionic groups at the cell surface. Dziemian (1939) has made an analysis of red blood lipoids which is suitable for this purpose. Some calculations based on this analysis are given in Table 5. The second column shows the fraction of the surface area of the red cells which will be occupied by the phospholipin if the phospholipin is uniformly distributed throughout the membrane. The calculation is a rough one based upon the proportion of phospholipin to triglyceride and sterol, using the cross-sectional areas of these molecules in monolayers given by Adam (1941). In the third column of the table is given the area per calcium atom assuming that two phospholipin molecules are required to bind one calcium atom. Should only one phospholipin molecule be required then these areas should be halved. In the last column of the

<table>
<thead>
<tr>
<th>Yeast</th>
<th>3 x 10⁻¹⁰ Å³</th>
<th>2.5 x 10⁻¹¹</th>
<th>3.8 x 10⁸</th>
<th>85 Å³²</th>
<th>10 Å³³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elodea</td>
<td>6.9 x 10⁻¹¹ Å³</td>
<td>5 x 10⁻¹³</td>
<td>7.5 x 10⁹</td>
<td>230 Å³</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Calculation from the results of Mazia (1940) of the area per calcium atom at the surface of various cells.
of the same ionic strength as blood plasma. The surface phase corresponding to these areas per table is given the concentration of calcium in the values obtained in the last two columns are, of course, maximal, since it has been assumed that all the phospholipin negative charge is neutralized by calcium, whereas in a physiologically balanced saline some would be balanced by sodium, potassium and magnesium, etc. In addition there will, no doubt, be a certain number of carboxyl groups from the protein molecules adsorbed on the surfaces of the red cells, which will contribute to the number of ions bound by the cell surface. The concentration of calcium obtained in this way for the rat red cell may be compared with the rough estimate for the same cell made in Table 3. The estimate made here is considerably the higher. This may be due to the factor of 10 taken for the ratio unionized: ionized calcium at the cell surface being too low, or to the electrophoretic mobility of the red cells being dominated by a protein layer adsorbed upon the lipoid layer, so that the electrophoretic mobility obtained is characteristic, not of the cell membrane, but of the adsorbed protein in the case of the results presented in Table 3 for red cells.

Table 5. Calculation of \([Ca]_a\) at the surface of red cells from the results of Dziemian (1939), assuming that one calcium atom is bound by every two phospholipin molecules

<table>
<thead>
<tr>
<th>Fraction of surface occupied by phospholipin</th>
<th>Area per Ca atom sq. A.</th>
<th>([Ca]_a) gm. mols per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>0.69</td>
<td>20</td>
</tr>
<tr>
<td>Rat</td>
<td>0.65</td>
<td>160</td>
</tr>
<tr>
<td>Beef</td>
<td>0.67</td>
<td>135</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.69</td>
<td>130</td>
</tr>
</tbody>
</table>

The distribution of the ions of any g-valent metal between the surface under consideration and the environment is given by the Gibbs-Donnan equilibrium between the surface and environmental phases:

\[
[M^{g+}]=r, \quad [M^{g+}]_b=r_b, \quad [M^{g+}]_a=[M^{g+}]_b, \quad \text{and} \quad [M^{g+}]_a=[M^{g+}]_b.
\]

where \(r\) is a constant for all metals. In addition, some of the metal will be forming an unionized complex with the anions of the surface; for this we may write the equation

\[
\frac{[M^{g+}]}{[A^-]_a} = (K)_a.
\]

If we could evaluate the various values of the equilibrium constant \((K)_a\) for this reaction we should be able to predict the relative toxicity of the different metals. Now if we ignore all specific relationships between the metals and the anions at the cell surface, and all specific characteristics of the metals other than those which enter into the standard electrode potentials of the metals, we can see a close relationship between the process of formation of a metallic ion at the electrode of the same metal, and the process of ionization of the unionized complex formed between the same metal and a given anion. In both cases the process consists essentially of the metal giving up one or more electrons, in the one case to the electrode and in the other to the potential (i.e. incipient) anion. The standard electrode potential, so that the toxic action of ions cannot due to such discharge of the resting potential. However, it has been found by J. R. E. Jones (1940) that Matthews’s prediction as to the relationship between standard electrode potential and toxicity is correct. For a number of organisms it was found that, for a given degree of toxic action, the logarithm of the concentration of the toxic metal in the environment is a linear function of the standard electrode potential. This remarkable relationship has not so far been given any theoretical foundation. But we have seen that for cells in their normal environment definite negative charges are present on the cell surface, probably consisting of carboxyl and phosphate groups. It is reasonable to suppose that the action of such ions as calcium is mediated by interaction with these negatively charged groups. It consequently would appear that if a sufficient proportion of these negatively charged groups were inactivated by formation of an unionized complex with a metal not normally present in substantial amount at the cell surface, abnormal behaviour would follow. Let us therefore assume that a given degree of toxicity is produced by conversion of a definite proportion of the anionic groups present at a vital surface of the cell into unionized complexes with the toxic metal. It is not necessary for our present purposes to specify further whether this surface is the surface of the cell or the surface of an enzyme, etc.

4. THE TOXICITY OF IONS

Matthews (1904) suggested that the toxicity of ions would be found to be proportional to their standard electrode potentials. His reason for making this suggestion was that he regarded the resting potential of a cell as being analogous to an electrode potential, in which case it would perhaps be discharged by ions in the same way as ions discharge an electrode potential. We now know that the resting potential of a cell is in many respects quite unlike an electrode
The biological action of ions and the concentration of ions at surfaces

Potential may be regarded (see Taylor, 1930) as a measure of the equilibrium constant for the reaction between metallic ions and electrons, whether this reaction takes place at an electrode surface or with a given anion. The equilibrium constant for the reaction in the case of hydrogen can be written in the form

\[ (K)_R = \phi e^{W/R \cdot T}, \]

where \( \phi \) and \( \beta \) are constants. Since the electrode potentials \( E_0 \) of the various metals are measured relative to the electrode potential of hydrogen, the equilibrium constant for any other metal \( M \) will have the form

\[ (K)_M = \phi e^{W+e\cdot E/R \cdot T} \]

where \( F = \) one Faraday, \( R = \) gas constant, \( T = \) absolute temperature. Then from equations (9), (10) and (12), we find that the total concentration of unionized metal complex at the surface is

\[ [MA_0] = \frac{r[M^{+n}]}{\phi} \cdot [A^-]^n \cdot e^{-W+e\cdot E/R \cdot T}, \]

This quantity will be constant for different metals if \([MA_0]\) is constant, and in this case \([A^-] = \) a constant and \( r = \) a constant also, at a given \( \rho H \) for ions of any one valency. Rewriting the above expression in logarithmic form we obtain

\[ 2.3 \log [A^-]+2.3 \log [M^{+n}], \]

or

\[ 2.3 \log [M^{+n}]+ \text{constant, for metals of a given valency.} \]

This last equation is the condition for obtaining solutions of ions of equal toxicity for ions of a given valency. If \([M^{+n}]\) is plotted against \( E_0 \), where \([M^{+n}]\) are equitoxic concentrations of different metals of the same valency, linear relationships will be obtained. This is the relationship obtained experimentally by Jones. The distance between the parallel straight lines found for the different valencies is given by the term \( 2.3 \log [A^-] \). In the poisoned condition \([A^-] \), and therefore \( r \), will probably be small. Consequently, the displacement between the parallel straight lines will be small and to a first approximation all the points may fall on one straight line. The results of Jones are in close accordance with this last prediction.

It appears from this examination that the toxic action of metal ions, in experiments such as those of Jones, is due to the formation of unionized complexes between anions present at an, at present, unidentified surface, and the metal ions.

5. THE UTILIZATION OF IRON
BY MARINE PLANTS

It has been shown by Cooper (1935, 1937) that the amount of iron present in the sea as free ions is extremely low, and is approximately \( 1.8 \times 10^{-18} \) g.mol./l. H. W. Harvey (1937) has shown that, if it is assumed that the rate of diffusion of iron into the interior of a plankton organism is the same as the rate of diffusion in sea water, the rate at which iron is taken up by a typical cell will be \( 1.6 \times 10^{-18} \) mg. of iron per day. If the cell is assumed to have a permeability of the same order of magnitude as that of other cells to ions, i.e. \( 10^{-18} \) g. atoms/\( \mu \) sec./mol./l., the concentration difference, it can be shown that for the same cell the rate of permeation is of the order of one atom of iron per day. The actual value which is found for the uptake of iron by such organisms is of the order of \( 8 \times 10^{-8} \) mg. of iron per day. This is far greater than the value which was calculated by Harvey, assuming the phytoplankton cell membrane to offer no more resistance to diffusion than an equal thickness of water, and enormously greater than the value calculated assuming the cell membrane has a permeability to ions of the same order of magnitude as that of other cells. It is therefore quite apparent that the cells cannot obtain their supply of iron as a result of simple penetration of the dissolved iron of the sea water into the cells by diffusion processes. However, as Cooper has pointed out, there is a considerable amount of iron present in the sea water as comparatively large particles of ferric hydroxide. Judging from the results of Hazel & Ayres (1931), ferric hydroxide in sea-water will be positively charged. In § 3 of this paper we saw that most cells are negatively charged at this \( \rho H \) and that as a result of this negative charge there is an excess of positive ions at the cell surface. For sodium ions this excess can be calculated from the formula

\[ \frac{[Na^+]_0}{[Na^+]} = r, \]

The corresponding formula for \( n \)-valent ions is

\[ \frac{[M^{+n}]_0}{[M^{+n}]} = r^n. \]

As is indicated by the results of Table 3, a common value for the ratio \( r \) is 2. Also the colloidal ferric hydroxide particles present in the sea may readily acquire a charge of 10 or 20 units. Inserting this into equation (15) we see that the concentration of iron at the cell surface as colloidal particles may easily rise to be \( 10^2 \) to \( 10^4 \) or more times greater than the concentration of colloidal iron in the sea water. On this view we should expect to find a high concentration of ferric hydroxide at the surface of phytoplankton: this ferric hydroxide will be available for combination with the anions of the cell surface and may readily be transported into the interior of the cell by any active process such as phagocytosis. These theoretical conclusions are in accordance with the experimental findings of Harvey (1937). He found that ferric hydroxide is readily adsorbed on the surface of diatoms, and that diatoms taken from the open sea have a large amount of iron upon their surfaces, far more than is required.
for continuous growth. He suggests that this iron may be adsorbed are able to act simultaneously. The chief mechanisms giving rise to adsorption of substances between surface and bulk phases due to charges fixed on the surface, as considered in earlier sections of this paper; (b) the adsorption of hydrocarbon groups at oil-water interfaces; (c) adsorption due to the interaction between the polar groups of a molecule and the polar groups of a surface; these interactions have recently been studied by Schulman & Rideal (1937). For a molecule such as adrenaline any one of these modes of adsorption will be insufficient to increase greatly the concentration of adrenaline at a surface. But if all three mechanisms can operate simultaneously, then a very considerable concentration of adrenaline may appear at the adsorbing surface. This adsorbing surface, to produce its effects, will need to have a unique steric arrangement so that the electrical charge on the

Table 6. Calculation of the increase in concentration of a substance at a surface having a specific structure adapted to the substance

<table>
<thead>
<tr>
<th>Substance</th>
<th>Formula</th>
<th>Energy of adsorption</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Electrostatic (Donnan)</td>
<td>CH₃ adsorption</td>
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<td>Adrenaline</td>
<td>OH OH OH CH₂CH₂NHCH₃</td>
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<tr>
<td>Hystamine</td>
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<td>2100</td>
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<td>Acetylcholine</td>
<td>CH₃CO₂CH₂CH₂N(CH₃)₃</td>
<td>700</td>
<td>4500</td>
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<tr>
<td>Choline</td>
<td>HO₂.CH₂CH₂N(CH₃)₃</td>
<td>700</td>
<td>3500</td>
</tr>
<tr>
<td>Atropine</td>
<td>CH₃CH₂CH₂CH₂N(CH₃)₂</td>
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<td>5000</td>
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<td>Ergot</td>
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<td>Ephedrine</td>
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<tr>
<td>Nicotine</td>
<td>N CH₄ CH₂NCH₃</td>
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6. THE ADSORPTION OF DRUGS, ETC., AT SPECIFIC SURFACES

Many physiologically active substances produce their effects under physiological conditions in concentrations of 1 x 10⁻⁸ or less. These substances, such as adrenaline and acetylcholine, must act upon the cell surface, or upon certain enzyme systems. In either case they must act within a surface phase, and it is somewhat difficult to see how the concentration of these molecules may rise to a sufficient value in the surface phase to exert a significant chemical effect. This difficulty may be to some extent surmounted if all the various ways in which a molecule may be adsorbed are able to act simultaneously. The chief mechanisms giving rise to adsorption in biological systems are: (a) the partition of ionic substances between surface and bulk phases due to charges fixed on the surface, as considered in earlier
The biological action of ions and the concentration of ions at surfaces

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SUMMARY

1. It is pointed out that at the surface of a cell the absolute concentration of ions, and the ratios of the concentrations of the individual ions, are usually different from the corresponding quantities in the environmental fluid. It is suggested that the necessity for a Na:Ca ratio of the order of 50:1 in a physiologically balanced fluid is found because such a ratio in the environmental fluid produces a Na:Ca ratio of the order of unity at the cell surface.

2. The concentrations of calcium and sodium ions at the surface of ovalbumin molecules are calculated. It is shown that calcium is bound (a) by electrostatic forces, (b) by formation of apparently unionized complexes with carboxyl groups. The complex formation obeys the law of mass action.

3. The ratio, total Na:total Ca at the surface of various cells in physiological media is calculated from the electrokinetic potentials of the cells. The calculated ratio is of the order of unity. In two cases calculation of the maximum possible total Ca at the cell surface by two other independent methods gives values of the same order of magnitude as the calculation from electrokinetic potentials.

4. It is shown that, where the toxic action of ions is due to complex formation with anions present at a surface, the logarithms of the equitoxic concentrations of the metals should be a linear function of the standard electrode potentials of the metals.

5. Calculations show that the concentration of colloidal Fe at the surface of organisms in sea-water should be far greater than the concentration in the environment; it is suggested that marine plants derive their iron from this layer of adsorbed colloidal iron.

6. A mechanism is suggested which provides for intense adsorption of drugs at specific surfaces.

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REFERENCES


